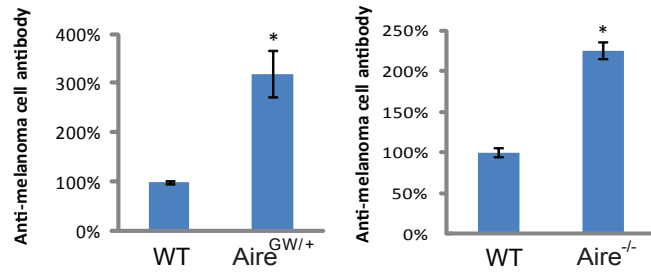
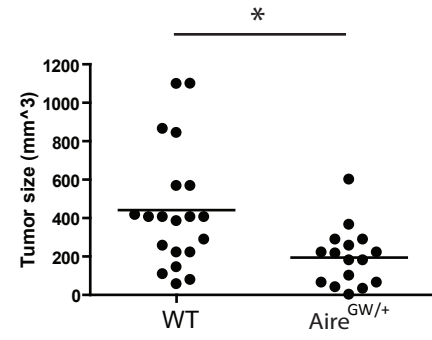


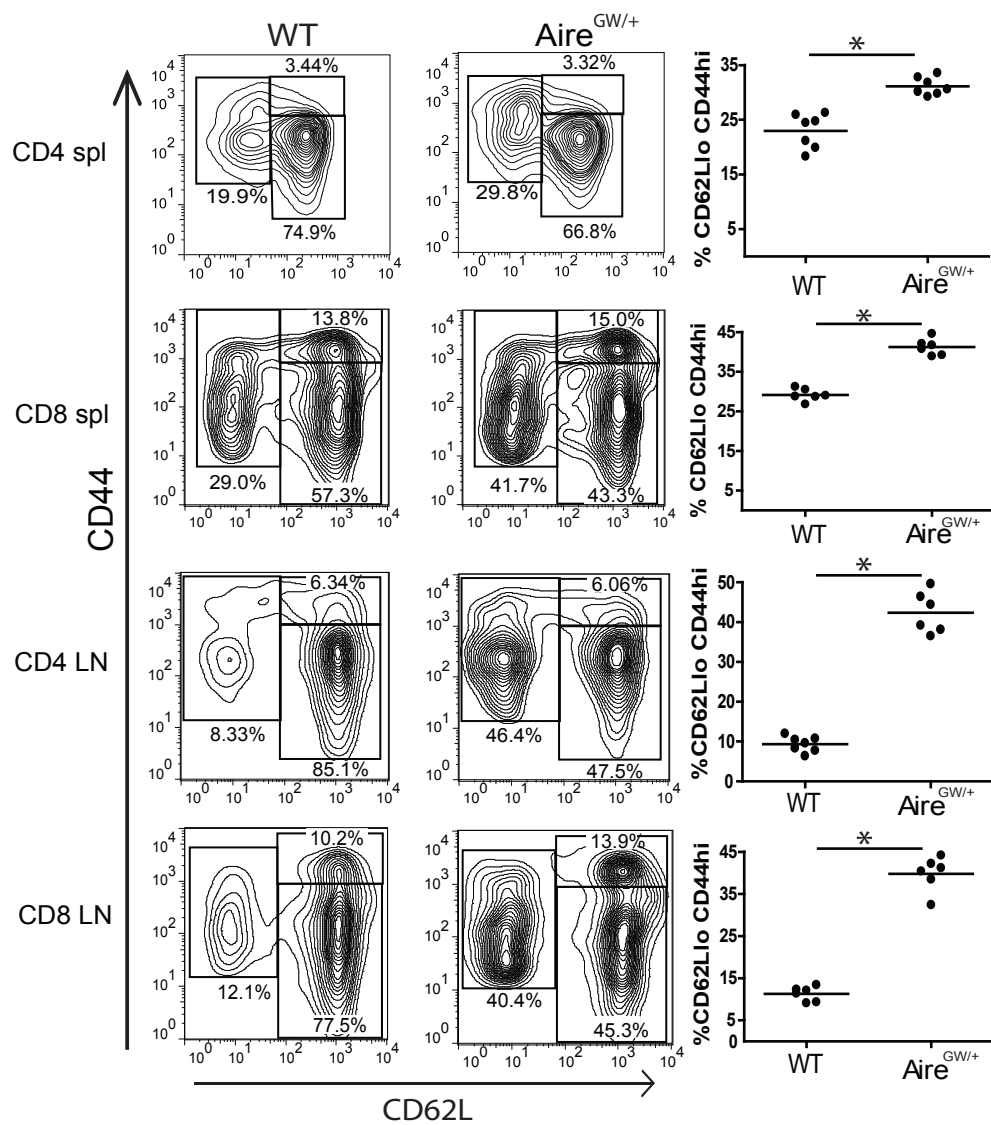
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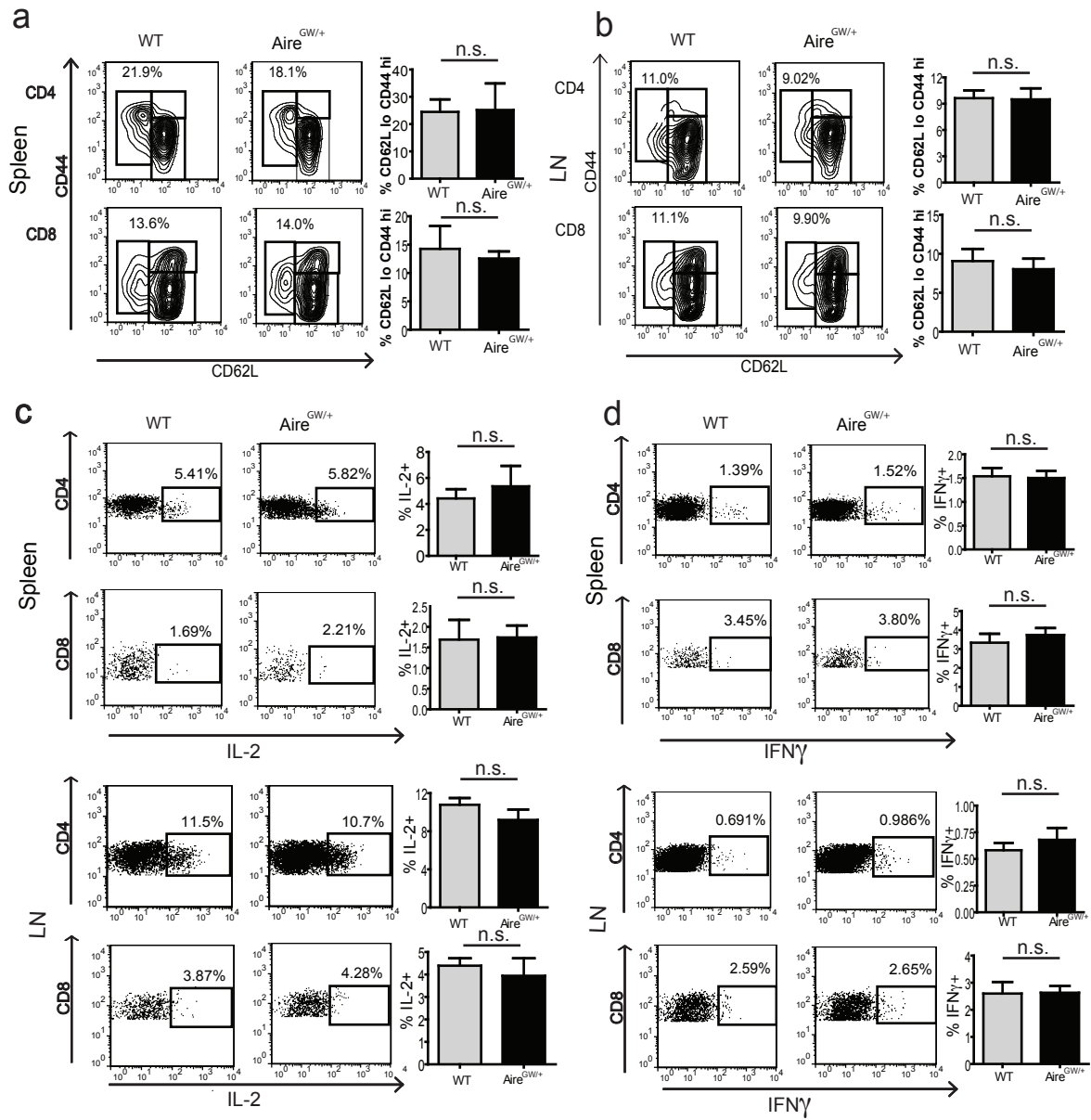
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Supplementary Figure 1

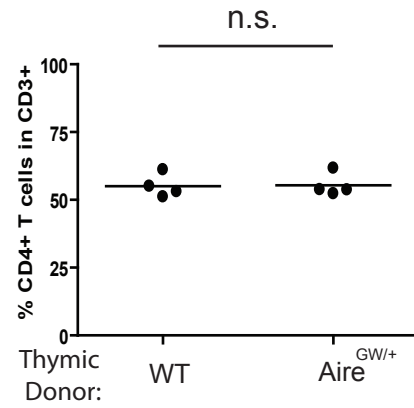
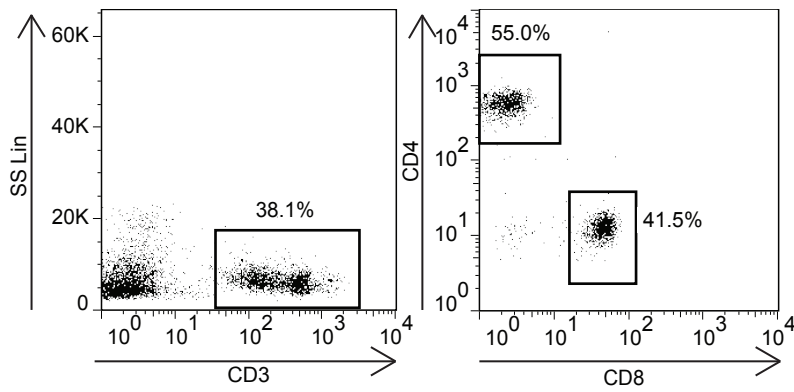


Supplementary Figure 2

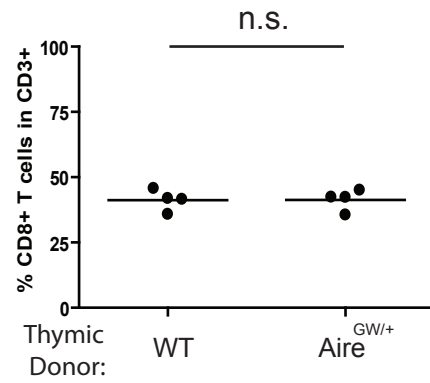
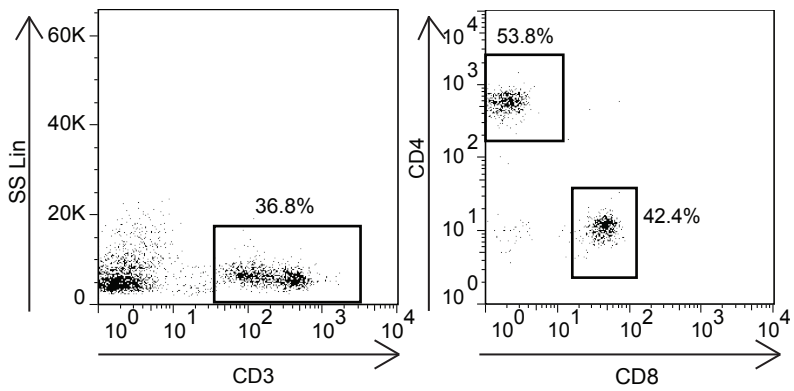


Supplementary Figure 3

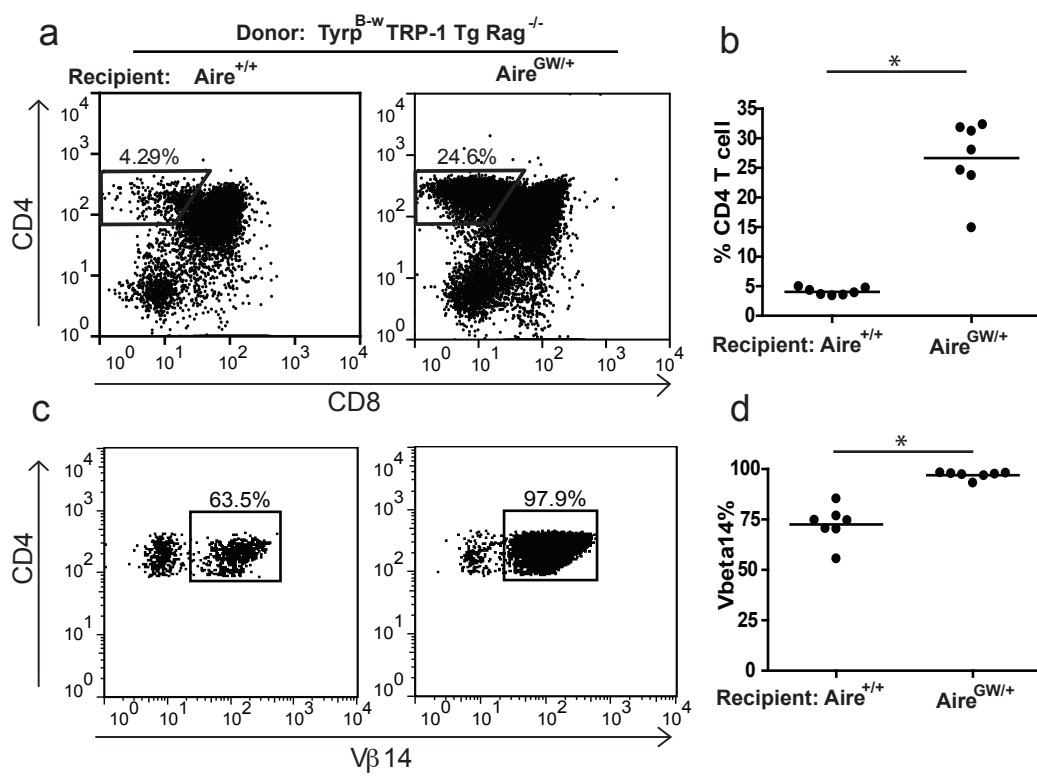
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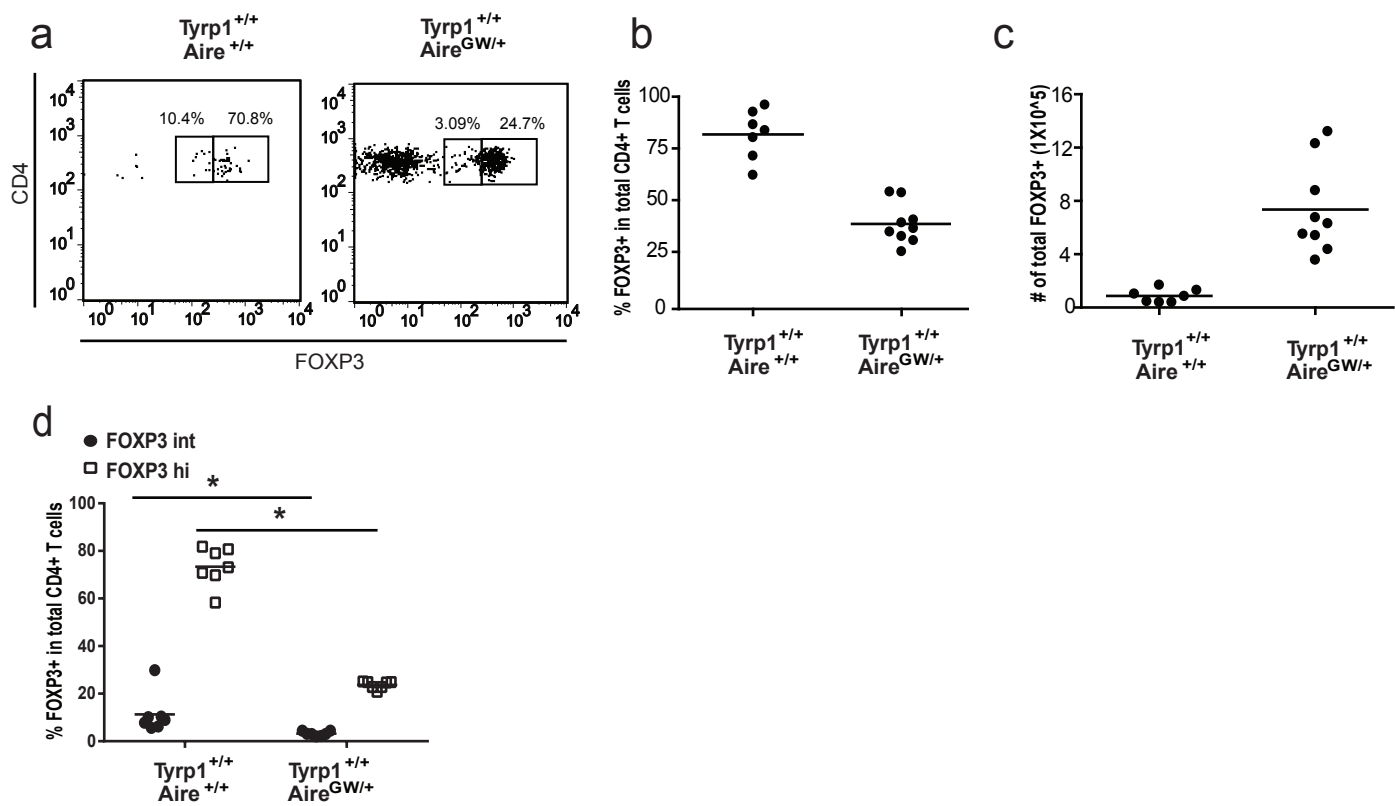
Thymic Donor: Aire^{GW/+}



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Supplemental Figure legends

Supplementary Figure 1. Increased autoantibodies to B16 melanoma extract and decreased B16 melanoma growth in Aire-deficient mice. (a) Relative amount of autoantibodies against B16 melanoma cell extract in sera from B6.Aire^{GW/+} (Aire^{GW/+}) mice, B6.Aire^{-/-} (Aire^{-/-}) or B6.Aire^{+/+} (WT) mice as evaluated by ELISA. Optical density values were normalized to WT levels, which were arbitrarily set to 100%. n=5 for each group, and 5 individual replicates were performed. (b) Tumor size at Day 16 post inoculation of B16 melanoma cells into either B6.Aire^{GW/+} mice or B6.Aire^{+/+} mice. Cumulative data from three independent experiments are shown. *P<0.05

Supplementary Figure 2. Increased frequency of activated/memory T cells in Aire-deficient mice inoculated with B16 melanoma cells. Representative flow plots of CD62L and CD44 expression in CD4⁺ and CD8⁺ lymphocytes from spleen and tumor draining lymph nodes (LN) of WT or Aire^{GW/+} mice inoculated with B16 melanoma cells. Cumulative data are shown in right panels. Each shape represents individual mouse. *P<0.05

Supplementary Figure 3. No change in T cell activation and cytokine secretion in Aire^{GW/+} mice spleen and lymph nodes. (a and b) Representative flow cytometry plots of CD62L and CD44 expression in lymphocytes from (a) spleen and (b) lymph nodes (LN) of WT or Aire^{GW/+} mice. Cumulative data are shown in right panels. n=4 for each group. (c and d) Representative flow cytometry plots of intracellular expression of IL-2 (c) and IFN- γ (d) from either CD4⁺ or CD8⁺ lymphocytes in spleen and lymph node. Cumulative data are shown in right panel. n=4 for each group. *P<0.05

Supplementary Figure 4. Reconstitution of T cells in B6 Nude mice transplanted with either WT or Aire^{GW/+} thymi. Leukocytes were isolated from peripheral blood of B6 Nude recipients transplanted with Aire^{GW/+} or WT thymi. CD3, CD4 and CD8 expression was evaluated by flow cytometry. Cumulative data are shown in right panels. Each shape represents individual mouse.

Supplementary Figure 5. Defective negative selection of TRP-1 specific T cells in Aire-deficient bone marrow recipients. (a) Representative flow cytometry plot of CD4 and CD8 expression in thymocytes from Aire^{+/+} or Aire^{GW/+} recipients of TRP-1^{B-w} TRP-1 TCR Tg bone marrow. (b) Percentage of CD4 SP thymocytes in Aire^{+/+} and Aire^{GW/+} mice. Each shape represents individual animal. (c) Representative flow cytometry plot of CD4 and Vβ14 expression in thymocytes from Aire^{GW/+} or Aire^{+/+} recipients of TRP-1^{B-w} TRP-1 TCR Tg bone marrow. (d) Percentage of CD4⁺ Vβ14⁺ thymocytes within the CD4 SP subset are shown. Each shape represents individual animal. *P<0.05

Supplementary Figure 6. Effects of Aire deficiency on TRP-1 Specific CD4⁺FOXP3⁺ Tregs in spleen (a) Representative flow cytometry plots of CD4 and FOXP3 expression in CD4⁺ splenocytes from Aire^{GW/+} TRP-1 TCR Tg RAG^{-/-} or Aire^{+/+} TRP-1 TCR TCR Tg RAG^{-/-} mice. (b and c) Cumulative data for percentage of CD4⁺FOXP3⁺ cells among CD4⁺ T cells (b) and absolute number of CD4⁺ FOXP3⁺ cells (c) in spleen are shown. (d) Cumulative data for percentage of CD4⁺FOXP3 intermediate (int) and CD4⁺FOXP3 high (hi) cells among total CD4⁺ T cells. Each shape represents an individual mouse. *P<0.05